

ENZYMATIC EXTRACTION OF *FICUS DELTOIDEA* FOR BETTER ENHANCEMENT OF EXTRACTED YIELD

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ABSTRACT

This thesis reported the enzymatic extraction of *Ficus deltoidea* (mas cotek) for better enhancement of extracted yield. *F. deltoidea* from Moraceae family which also known as “mas cotek”, “serapat angin” and “telinga beruk” in Malaysia, “tabat barito” in Indonesia and “ kangkalibang” in Africa. This plant has many used in traditional medication such as to help uterus contraction after deliver, improve blood circulation, reducing cholesterol, treat diabetes and also can use as aphrodisiac. Previous research had shown that there were several active compounds in *F.deltoidea* which are flavonoids, phenol, tannis, riterpenoids, isovitexin, vitexin, proanthocyanidins, flavan-3-ol monomers and flavones glycosides. Since the uses of active compound of this plant have been known and have the high demand, there are several extraction technique have been research. However, the ideal technique is not reported yet. Hence, this work aims to study the most ideal extraction technique of *F.deltoidea*. The *F. deltoidea* ground leaves are added into the water to form suspension. The ground leaves are extracted by using two methods which is hydro distillation which is extraction by using water only and enzyme cellulase extraction which is the extraction wit enzyme assist. For the first enzymatic extraction, the different concentrations of enzyme cellulase but same temperature which is 50⁰C are used to get the optimum concentration. For the second enzymatic extraction used different temperature from the optimum concentration. The suspension is separated to oil-rich and emulsion phase by using centrifuge. The extraction is withdrawn by using micropipette. Then, the extracted active compound is analyzed by using high performance liquid chromatography (HPLC) to identifying and purifying the active compounds of the mixture. The isovitexin is used as the standard which is the active compound that will be detected by HPLC. According to the HPLC analysis, the retention time of isovitexin detected is around 13 min to 14 min for the standard solution. The optimum concentration for enzyme extraction is 1 mg/L. The yield of the enzymatic assist is higher than the control extraction which is without enzyme.

ABSTRAK

Tesis ini melaporkan tentang pengekstrakan *Ficus deltoidea* (mas cotek) dengan menggunakan enzim bagi meningkatkan lagi hasil diekstrak. *F. deltoidea* tergolong dari kumpulan Moraceae yang juga dikenali sebagai “mas cotek”, “serapat angin” dan “telinga beruk” di Malaysia, “tabat barito” di Indonesia dan “kangkalibang” di Afrika. Tumbuhan ini banyak digunakan dalam ubat-ubatan tradisional seperti membantu pengecutan rahim selepas bersalin, meningkatkan peredaran darah, mengurangkan tahap kolesterol, merawat kencing manis dan juga boleh digunakan sebagai afrodisiak. Kajian sebelum ini telah menunjukkan bahawa terdapat beberapa sebatian aktif yang terkandung di dalam *F. deltoidea* iaitu flavonoid, fenol, tanis, riterpenoids, isovitexin, vitexin, proanthocyanidins, monomer flavan-3-ol dan flavon glikosida. Sejak kegunaan sebatian aktif tumbuhan ini diketahui ramai disamping mempunyai permintaan yang tinggi, beberapa penyelidikan tentang pengekstrakan telah dijalankan. Walau bagaimanapun, teknik pengekstrakan yang ideal belum dilaporkan lagi. Oleh itu, kajian ini bertujuan untuk mengkaji teknik pengekstrakan yang paling sesuai untuk mengekstrak daun *F. deltoidea*. Daun *F. deltoidea* yang telah dikeringkan dan telah dikisar dimasukkan kedalam air untuk menghasilkan campuran. Daun ini diekstrak dengan menggunakan dua kaedah iaitu hydro-penyulingan iaitu pengekstrakan yang menggunakan air sahaja dan pengekstrakan menggunakan bantuan enzim. Pengekstrakan menggunakan enzim yang pertama ialah menggunakan kepekatan enzim yang berbeza tetapi suhu yang sama iaitu 50⁰C dan mendapatkan kepekatan yang optimum. Yang keduanya ialah menggunakan kepekatan enzim yang optimum tetapi suhu yang berlainan. Campuran dipisahkan menggunakan centrifuge. Pengekstrakan itu diambil menggunakan micropipette. Sebatian aktif yang diekstrak kemudiannya dianalisis menggunakan kromatografi cecair prestasi tinggi (HPLC) untuk mengenal pasti sebatian aktif. Isoviteksin digunakan sebagai standard yang merupakan sebatian aktif yang akan dikesan oleh HPLC. Menurut analisis HPLC, masa untuk mengesan isovitexin adalah dalam sekitar 13 min ke 14 min. kepekatan optimum untuk pengekstrakan adalah 1 mg/L. pengekstrakan menggunakan bantuan enzim adalah lebih berkesan daripada tanpa bantuan enzim.

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LIST OF ABBREVIATIONS

<i>F.d</i>	figus deltoidea
t_R	retention time
<i>V</i>	volume
v/v	volume per volume
<i>w</i>	weight of sample
w/v	weight per volume
<i>y</i>	yield

LIST OF ABBREVIATIONS

ACN	Acetonitrile
HPLC	High performance liquid chromatography
MARDI	Malaysia Agriculture Research and Development Institute
rpm	Revolutions per minutes

1 INTRODUCTION

1.1 Background

Medicinal and herbal plants are very important nowadays and also have been used since a long time ago in Malaysia. The demands for the herbal plant are always increase due to the increasing in the usage. Basically, there is high antioxidant activity that contained in herbal and vegetable plant (Asmah *et al.*, 2003). It is very important in inhibiting and scavenging radicals and also provide shield to humans against treat such as infections and degenerative diseases. The effective antioxidants from natural sources are strongly needed as alternatives to synthetic food additives in order to prevent deterioration of foods, drugs, and cosmetics for their role in the maintenance and improvement of health and wellness. In this research, there are also antioxidant activities in the herbal plant which is Mas Cotek.

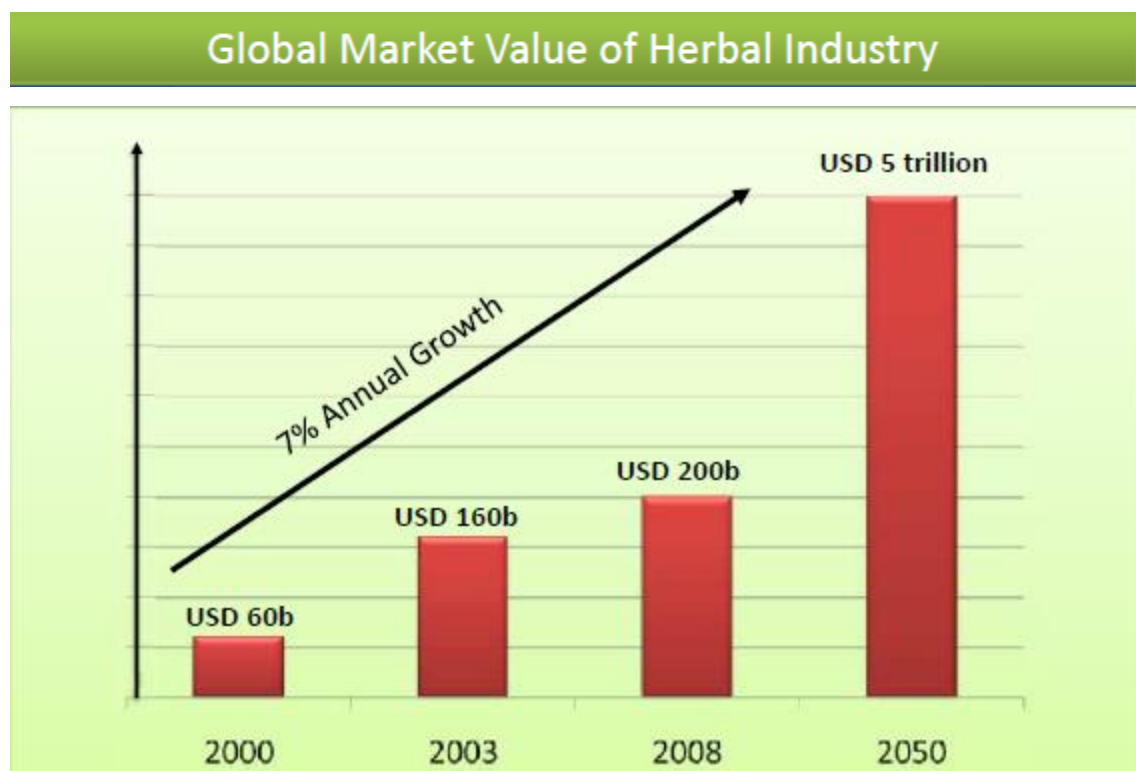


Figure 1-1: Global Market Value of Herbal Industry according to year

Mas Cotek or known as *Ficus deltoidea* in the scientific are from Moraceae family (Mahmood *et al.*, 2010). The name of Mas Cotek is well known in Malaysia and there also several others name such as 'serapat angin' and 'telinga beruk'. While, in Indonesia they known as 'tabat barito' and in Africa known as 'kangkalibang'. Unlike the *Labisia pumilla* (kacip Fatimah) which specifically used for woman and *Eurycomo longifolia* (tongkat ali) which specifically used for man, *F. deltoidea* didn't have the specification of uses whether for both man and woman (Hakiman and Maziah, 2009).



Figure 1-2: *Ficus deltoidea* tree

According to the research by Malaysia Agriculture Research and Development Institute (MARDI), there are several active compounds that contain in *F. deltoidea* and the main four are flavonoid, tannis, triterpenoids, and phenols. The herbal plant can be used in many industries such as flavor and fragrance, pharmaceuticals and herbal remedies. Since there is strong antioxidant that contain in *F. deltoidea* which is in phenols, it can be used in all of those industry but mostly *F. deltoidea* are used in pharmaceuticals and herbal remedies.

Plant cell wall consists of cellulose, hemi-cellulose and pectin which act as the barrier for the intracellular substance to be release. Degradation of the cell wall is the basic step to release the active compounds. Usually, the active compounds are extracted by using extraction process which is transferring the solutes from a solid medium to a solvent. There are various methods that been used to assist the extraction of the active compound from plant such as ultrasonication, supercritical fluids, microwave, membrane adsorption and molecular imprinting. However, there still some active compound that still retain in the polysaccharide-lignin network by hydrogen or hydrophobic bonding,

which cannot be easily extracted by solvent. Thus, Chen *et al.* (2011) suggests that enzyme-assisted extraction, in which the extraction is improved the cell wall degradation. This method also can increase the solubility of the target compounds in the extractant.

Extraction of natural functional compounds from plant by using enzyme-assisted is widely investigated in recent years. The advantages for this method are can provide high efficiency, easily operated, and environment friendly. The enzymes breaking down the cell walls and the membrane of the plant and this will enable the extraction of active compound to better release. This method also can reduce the usage of solvent and can lower the energy consumption. Thus, enzymatic-assisted extraction has been represented as an alternative method to extract the natural product.

In past, the conventional extraction method of *F. deltoidea* required long extraction time and high energy consumption with low extracted yield. Therefore, to increase the extracted yield, the ideal extraction method should be introduced.

1.2 Problem statement

In increased of health problem and rising health concern in modern world has promoted various medicinal and natural products. In Malaysia especially, there are many causes of death and the major cases are heart attack and the major sickness for Malaysian are diabetes. The active compound that contain in *Ficus deltoidea* can be used to reduce the cholesterol in the body and also will increase the blood circulate. Thus, the demand for the *F. deltoidea* extraction increased due to increasing the health concern in human.

The active compound in *F. deltoidea* can be better release by breaking down the cell wall of the plant. *F. deltoidea* which is the lignocellulosic material that contain high ligning and hemicellulosic that cannot be break easily. Thus, the enzyme is used to assist the solvent to break down the cell wall and the membrane of the plant.

Besides, by using enzymatic extraction will reduce the cost of the process and the time to produce the oil yield. The other method of extraction such as using water and solvent extraction need more process cost and also takes the long extraction time to produce the oil.

1.3 Objectives

The following are the objectives of this research:

- To extract active compound of *F. deltoidea* using enzymatic extraction as the main method.
- To compare the result which in process time, yield and extraction productivity for extraction with other extraction methods.

1.4 Scope of this research

In order to achieve the objective, the following scopes have been identified and to be applied:

- i) Optimization study of enzymes for *Ficus deltoidea* extraction.
- ii) Use enzymatic extraction to extract active compound of *Ficus deltoidea*.
- iii) Analyze the active compound constituents from the method using HPLC.
- iv) Compare the yield of active compound of *F. deltoidea* from the method.

1.5 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:

In chapter 2, it is involves about the overall explanation about the *Ficus deltoidea* including history, habitat, tree and industrial development. Besides, the uses and the active compound that contain in *F. deltoidea* also mentioned in this chapter. More than that, the different types of methods of extraction such as water extraction and enzymatic extraction also mentions. Everything about all these and the statement the previous research are also presented.

Chapter 3 gives the explanation about the method used in this research. All the methods involve such as preparation of raw material and extraction process which is using water extraction and enzymatic extraction. Besides, analytical methods are also provided in this chapter. In addition, all the parameters related in this research are also described as it is necessary.

Chapter 4 will be present about the result got from this research. The result gained from this research is explained.

Chapter 5 draws together a summary of the thesis and outlines the recommendation for the future work which might be derived from the model developed in this work.

2 LITERATURE REVIEW

2.1 Overview

This chapter are about the finding of the related article from previous researches about the history, habitat, industrial development, usage, and active compound that contain in *Ficus deltoidea*. This chapter also tell about the extraction technique that be used by previous researches.

2.2 *Ficus Deltoidea*

2.2.1 History, habitat and industrial development

Ficus deltoidea or known as Mas Cotek is belongs to Moraceae family. This plants are originated in Asia Minor and can be found throughout Mediterranean, India subcontinent, Latin America, Texas, Southern California, until the far east such as Malesian tropical rain forest which about 750 species (Nashriyah *et al.* 2012). It is one of the largest genres of flowering plants with six traditional subgenera that recognized based on morphology and distribution. Due to the diverse natural habitats, the *Ficus deltoidea* plant successful spreading to the worldwide. According to Nashiriyah *et al.* (2012), *Ficus deltoidea* mistletoe fig is a native of Peninsular Malaysia and been introduced elsewhere which is in Malesia that include Thailand, Indonesia and Malaysia.

According to Mahmood *et al.* (2010), the vernacular name for *F. deltoidea* are ‘mas cotek’, ‘serapat angin’, ‘telinga beruk’ and many more in Malaysia, while in Indonesia known as ‘tabat barito’, and in Africa known as ‘kangkalibang’. There are fine spots with gold colour on the surface of each leaves and the vernacular name of Mas Cotek is given in Peninsular Malaysia because of it.

Ficus deltoidea plant is commonly found in Peninsular Malaysia which is generally as an epiphyte; depend on another bigger or taller plant (usually 20 feet from the land) to reach their own sunlight resource, in lowlands and mountains but also as a terrestrial shrub on sandy shores, mountain tops and in bogs (Starr *et al.*, 2003). The majority of varieties grow at below 1200 m altitude, however *F.deltoidea* var. *intermedia* can be found in the higher mountain areas above the dipterocarp forest. *Ficus deltoidea* var.

angustifolia grows in places by the streams or riversides. In Borneo, the var. *motleyana* is found in the coastal, peat-swamp and sandy heath forest.

Ficus deltoidea have two types namely the male and female. The male and female plant can be differentiated by their morphology. The male plant has smaller and elongated leaves size whereas the female plant has larger and more rounded leaves (Naquib & Vivi, 2013). The top of the leaf surface is shiny green in colour while golden in color (rust-red to olive brown) with black dots under the leaf (Starr *et al.*, 2003). Normally, the flowers are found freely in paired or single for each plant. The flower is built in receptacle where the fruits and flowers are appearing inside. The colour of flowers changed from green to red when it became matured.



Figure 2-1: Comparison between male and female leaves of *Ficus deltoidea*

The herbal extractions have many uses. Their uses can be channeled into four product groups which are herbal remedies, pharmaceuticals, flavor and fragrance and bio pesticides. According to Rasadah & Li (2008), herbal medicinal products have taken on increasing significance as regards medical and economic importance over the last two decades, and have registered as strong comeback in developed countries in the USA, the European Union (EU), Australia and Canada. The global expansion of the herbal industry has a significant impact on the Malaysia market. In Malaysia, the herbal market which was worth RM3.8 billion (USD 1.03 billion) in 2006, is anticipated to grow with an annual growth rate of between 15 and 20 percent.

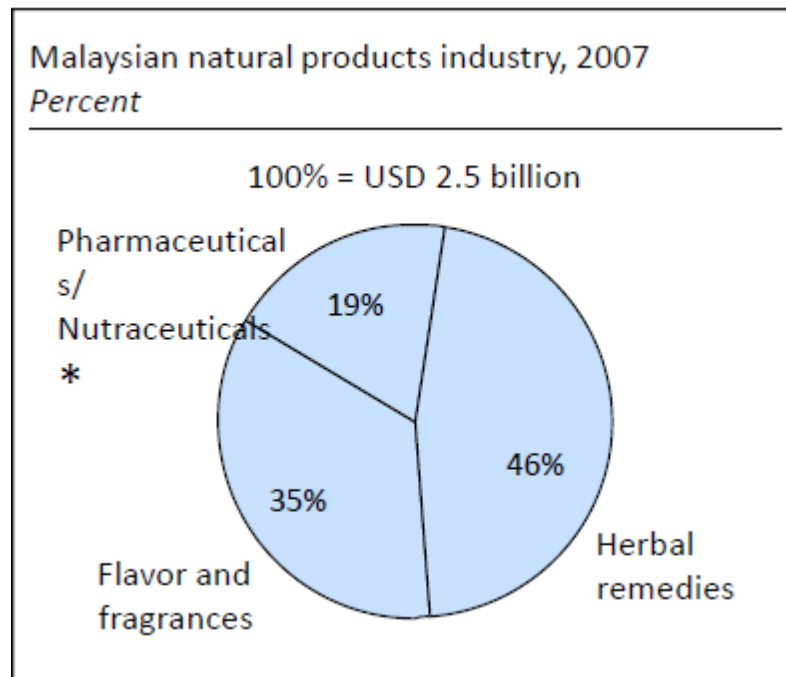


Figure 2-2: Malaysian natural product industry in 2007

There is a focus on herbs based on traditional systems of medicine and remedies based on folk knowledge due to their access, low cost and faith of the people. Natural products from medicinal and aromatic plants can be applied to a wide range of items such as herbal and traditional medicine, food and beverages, bio-pharmaceuticals, flavours and fragrances, cosmetics and toiletries, dye, detergents, bio-pesticides and special chemicals. Considering their importance, quality is essence and guidelines for quality control at each step of production are critical to the sustainability of the product and its wide acceptance. For *Ficus deltoidea*, it commonly used in herbal and traditional medicine, bio-pharmaceuticals, and cosmetics and toiletries.



Figure 2-3: *F. deltoidea* based product

2.2.2 Benefits of *Ficus deltoidea*

According to the research that been made by Malaysia Agriculture Research and Development Institute (MARDI), *F. deltoidea* contains antioxidant chemicals such as saponins, flavonoids, tannins, polyphenols, triterphenoids, and proanthocyanins. The analyses of the chemical and nutritional constituents are summarized in Table 2.1 below.

Table 2-1: Chemical and nutritional constituents of *F. deltoidea*

Chemical	Nutritional constituents
Naringin	An anti-inflammatory for small injury.
Flavonoid	Acts an antioxidant which affects better blood flow, antiviral and as heart and estrogen hormones protector.
Tannins	Protect the skin and acts as an anti-wrinkle, diarrhea and protect the damaged tissue.
Polyphenols	Young leaves contain high polyphenol that acts as anti-inflammatory and anti-septic.
Triterpenoids	Important during the nutrient absorption process.
Proanthocyanins	Protect tissues from damage and it also acts as anti-toxic.

Other than that, a researcher from Universiti Malaya reported that a right combination of *Ficus deltoidea* species is capable to neutralize body system. Therefore, *F. deltoidea* can reduce high blood pressure problem, diabetes, gout, migraine, ulcer and other diseases.

According to Adam *et al.* (2009), *F. deltoidea* has been claimed to have antidiabetic properties and has been used as remedy to treat the diabetes. This theory is based on the ethnobotanical approaches and the scientific evidence to confirm its efficiency however is still lacking. Zainah *et al.* (2010) have done the research on antihyperglycemic and glucose tolerance activity of *Ficus deltoidea* on diabetic rats by using ethanolic extraction. In the paper, 70% ethanol are used to extract ground *F. deltoidea* leaves for 3 days at room temperature. The result show that the *F. deltoidea* extract can reduce the blood glucose level in the diabetes rats. As the result, while using the 500 mg/kg/b.w of extract *F. deltoidea* the glucose level in the blood decrease from 23.81 ± 0.98 mmol/L to 21.21 ± 2.48 mmol/L for 2 hours. Adam *et al.* (2010) also do the research on the reducing blood glucose and the result shows that the blood glucose reduced at the certain time. Besides, according to Mahmood *et al.* (2010), *Ficus deltoidea* extract also can be used to heal the wound. The group rats are taken and treat by using sterile deionized water, blank placebo and placebo containing *F. deltoidea*. From the result, wound treat with aqueous extract *F. deltoidea* showed considerable signs of dermal healing and significantly ($p < 0.05$) healed earlier compared to wounds that treat with sterile deionized water or blank placebo.

2.2.3 Traditional uses of *F. deltoidea*

According to Malaysia natives, the *Ficus deltoidea* plant is usually used by the women that just give birth, regain energy, repair blood flows and associated problem and also can be used as herbal drinking for healthy and beauty. For almost traditional medicine practice, *F. deltoidea* is also capable of preventing and curing certain diseases if it is used rightly. For example, *F. deltoidea* can be used to cure diabetes (Adam *et al.*, 2009), high blood pressure and low libido energy.

According to the natives also, these herbs must be boiled with the water for 45 minutes until all the extracts whether from the stems, leaves, fruits, twigs or roots are obtained. Then the extracts that obtain from boiling are filtered. It can be stored in refrigerator for

longer use but must be consumed within 3 days. This drink is suitable for everyone with no limits of age and genders; however it is not suitable for pregnant women.

2.3 Antioxidants

The antioxidant activity and its potency in certain plant depend on the existence of various compounds found in that plant. Some of phenolic compounds (anthocyanidin, catechines, flavones, flavonols and isoflavones), tannins (ellagic acid, gallic acid), phenyl isopropanoids (cafeicicis, coumaric acid, ferulic acid), lignans, catchol and others are antioxidants. The antioxidative and radical scavenging activities of this antioxidant are well studied (Emami *et al.*, 2007).

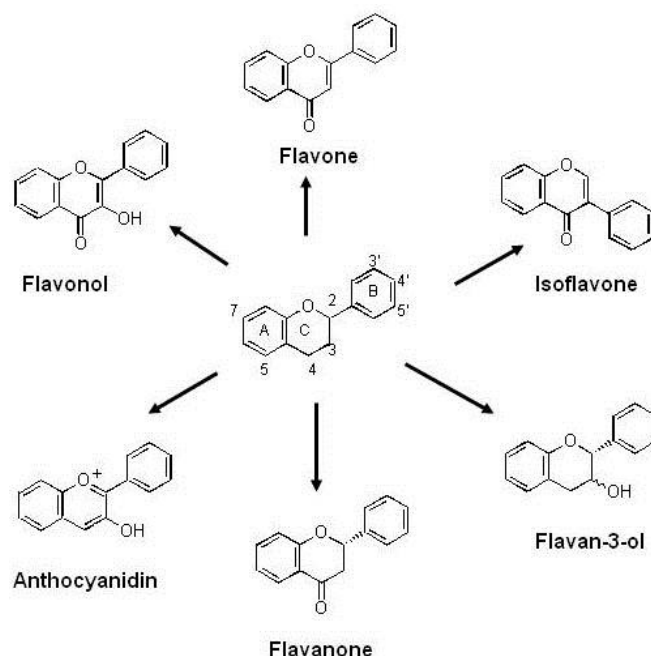


Figure 2-4: Basic structure of flavonoids

Antioxidant inadequacy is associated with oxidative damage or kills to DNA of the germ line as well as somatic cells. In addition to the reduction to the reduction in cancer rates, high consumption of antioxidants is related to reduce risk of cardiovascular disease including heart attack and coronary heart disease. Although antioxidant supplementation is widely used in order to avoid the development of cancer, it has been suggested that antioxidants may interfere with cancer treatments. High doses of water-soluble antioxidants such as ascorbic and very high doses of some antioxidants are found to be toxic. This may have adverse long-term effects.

2.4 Extraction of active compound

Plant cell wall consists of cellulose, hemi-cellulose and pectin which act as the barrier for the intracellular substance to be release. Degradation of the cell wall is the basic step to release the active compounds. Usually, the active compounds are extracted by using extraction process which is transferring the solutes from a solid medium to a solvent. There are various methods that been used to assist the extraction of the active compound from plant such as ultrasonication, supercritical fluids, microwave, membrane adsorption and molecular imprinting. However, there still some active compound that still retain in the polysaccharide-lignin network by hydrogen or hydrophobic bonding, which cannot be easily extracted by solvent. Thus, Chen *et al.* (2011) suggests that enzyme-assisted extraction, in which the extraction is improved the cell wall degradation. This method also can increase the solubility of the target compounds in the extractant.

2.4.1 Water extraction

The water extraction method is a method that commonly used in chemistry for general extracts of interest extraction. This process used a combination of high pressure water for agitation and hot water to increase reaction rate. The process generally involves taking a mixture of substances; dissolve in warm water and then cooling the mixture. The insoluble compounds precipitate out of the water, while the soluble ones stay dissolve. The solution can be separated by filtration or decantation. This process works by exploiting the differences in solubility (with respect to temperature) of varying substances.

Mostly of the report are used water and solvent to extract the bioactive compound. Misbah *et al.* (2013) and Adam *et al.* (2010) used distilled water to extract the bioactive compounds. While Farsi *et al.* (2011) used solvent to extract the bioactive compound. Some of active compound generally have poor solubility in mild solvents such as ethanol-water solution. Therefore, to increase the extracted yield, the ideal extraction method should be introduced.

2.4.2 Enzyme assisted extraction

Extraction of natural functional compounds from plant by using enzyme-assisted is widely investigated in recent years. The advantages for this method are can provide high efficiency, easily operated, and environment friendly. The enzymes breaking down the

cell walls and the membrane of the plant and this will enable the extraction of active compound to better release. This method also can reduce the usage of solvent and can lower the energy consumption. Thus, enzymatic-assisted extraction has been represented as an alternative method to extract the natural product.

There are several reports that study on enzyme-assisted extraction for bioactive compound. Chen *et al.* (2011) has been study on enzyme-assisted extraction of flavonoids from *Ginko biloba* leaves. From the study, found that enzyme was the effectively hydrolyze the active compound. Although the enzyme-assisted approach has largely improved the extraction rate, but the solvent such water still needed to perform the extraction after enzymatic treatment.

2.5 Summary

Nevertheless, regarding toxicological limitations, there is a clear trend in the industry to substitute these organic solvent for alternative nontoxic solvents, with the first option by using water or alcohols. Therefore, large amount of high concentration ethanol-water solution is often used. However, this will leads to high production cost, making it desirable to increase the solubility of active compound without affecting their physiological activities.

3 MATERIALS AND METHODS

3.1 Overview

In this chapter, we are discussing more detail about the method to conduct in this research on enzymatic extraction of *Ficus deltoidea*. The procedures include in this research are, preparing the *F. deltoidea* powder and extraction process by using water extraction and enzyme extraction. There are also including the analytical method in which the method to determine the yield of the extracted essential oil from *F. deltoidea* leaves.

3.2 Introduction



Figure 3-1: Process flow

The first step of the experiment is preparing the *Ficus deltoidea* powder which is the plant leaves need to be dried at certain temperature and then grind to form the powder. *Ficus deltoidea* need to be dried to remove all the moisture content in the leaves.

After that, the *Ficus deltoidea* powder must be immerse into the solvent to do the extraction process. In this research, there are two types of extraction process which is by using hydro distillation extraction process and enzymatic assisted extraction process. There is another method of extraction that using chemical solvent that known as solvent extraction like methanolic and ethanolic extraction. Hydro distillation is used for this experiment because of its environmental friendly and same goes to enzymatic extraction. There is not chemical as a solvent involve in the extraction of active compound. Other than that, both of these methods are used a lower production cost and very simple extraction method.

After the production of essential oil, high performance liquid chromatography is used for the composition analysis. In this step, the data were collected and the yield will be calculated.

3.3 Chemicals

The phosphoric acid and acetonitrile are obtained from Sigma- Aldrich Malaysia. Enzymes which is cellulase from *Aspergillus niger* used in the study are also obtained from Sigma- Aldrich Malaysia. The technique cell free enzyme is conducted during the experiment.

3.4 *Ficus deltoidea* leaves

The original leaves of *F. deltoidea* are obtained from Sungai Tinggi Selatan, Selangor, Malaysia. The leaves are dried in the oven at temperature 45⁰C and grind into the fine powder. Then, the powders are stored until experiment.

3.5 Hydrodistillation extraction

The powder from *F. feltoidea* leaves (100 g) will through hyrodistillation extraction process for 10 hour and the distillate be collected every 2 hours.

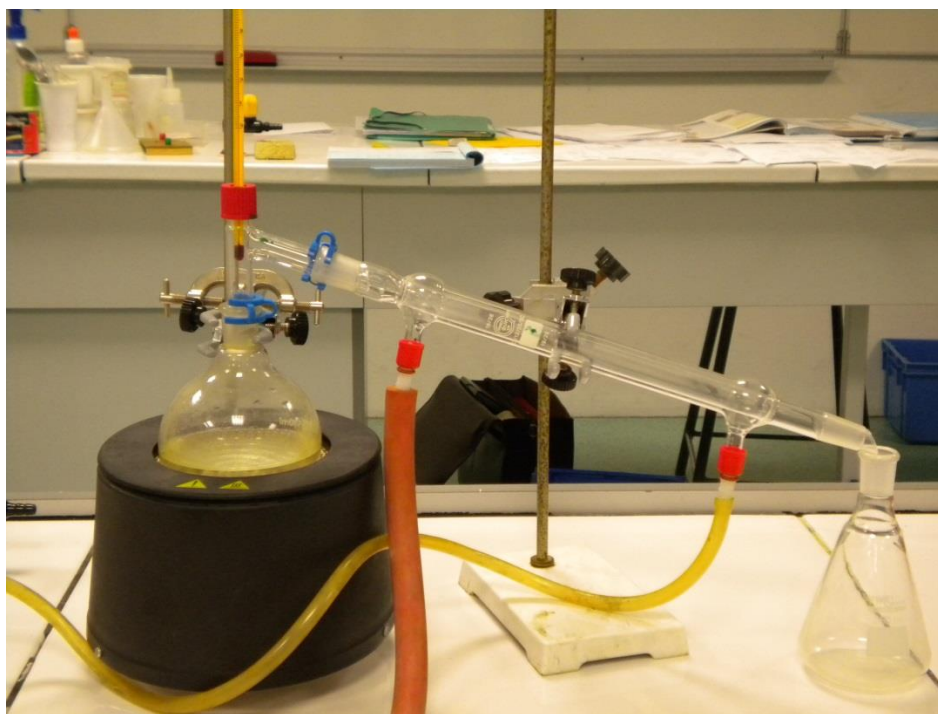


Figure 3-2: Hydrodistillation process